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# Some toxic and antitoxic effects in cultures of Spirogyra *

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In view of difficulties commonly experienced in maintaining algal cultures in the laboratory, it seemed desirable to make an experimental study of some of these difficulties, as was suggested to the writer by Professor Georg Klebs, and the present publication deals with a portion of such a study. The experimentation was carried out in the Botanisches Institut at Heidelberg in 1909 and 1910, the facilities of these laboratories being made available through the kindness of Professor Klebs. The experiments to be considered below bear upon the preparation of a nutrient solution suitable for algal growth under laboratory conditions, and undertake a partial analysis of the relations of the aqueous medium to the success or failure of algal cultures in glass.

The alga used for this study was *Spirogyra longata* (Vauch.) Kg.,† brought from Algiers in the spring of 1906 and kept since then (until the winter of 1909–10) in a north window of the Botanisches Institut at Heidelberg, with only small additions of tap water from time to time. At the time of beginning these studies the culture was healthy and showed vigorous growth, and the material seemed excellently suited for experimentation of the sort in hand; all the filaments had developed under closely similar conditions and were obviously adjusted to the conditions existing in the laboratory. In the experimentation it was thus possible to alter the culture medium without greatly changing the other environmental factors—*e. g.*, light, temperature—under which the plants had developed. The last consideration is an important one; results obtained with material brought from various other conditions to the laboratory cannot be interpreted as caused by changes in the nature of the culture solution alone, a point well emphasized by some of the considerations to be brought out below.

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* Botanical Contribution from The Johns Hopkins University, No. 29.

† Determined from Einfachste Lebensformen des Tier- und Pflanzenreiches, Eyferth, B., 3 Aufl. 1900.

The method here followed was to transfer a few (about 30 to 50) filaments from the stock culture to about ten cubic centimeters of the medium to be tested, in a covered glass dish. These transfers were made late in the afternoon, and the cultures were subjected to microscopic examination, without removal from the culture dishes, on the next morning and on succeeding days until final results were obtained. The experiments lasted for periods of from one to eighty-six days. The condition of each culture was recorded in the following terms. *Excellent* means that, at most, only a few cells showed injury; *good,* that considerably more than half of the filaments seemed uninjured; *poor,* that more than half were injured; *dead,* that practically every cell was dead. The tables used in this paper have been constructed from the daily records by stating in similar terms the general condition of the cultures throughout the experiments.

Precautions were of course taken to have the dishes and instruments as clean as possible. Any dish once used for a poisonous solution was discarded at the end of that experiment, and dishes were sometimes interchanged and furnished with fresh supplies of the media to be tested. The different lots of special distilled water did not enter into experiments until they had been found to be harmless to the plant here used. In most cases about the same amounts of media were used in the different experiments; the quantity of solution as related to the number of cells in any culture is to be considered as an important factor in all experiments bearing upon the relation of the nature of the medium to the behavior of organisms existing therein.

## I. NUTRIENT SOLUTIONS

The nutrient media used were all prepared according to the formulae given by Küster (10), being those of Sachs, Knop, Molisch, and Crone.* These were afterward diluted to the required concentration with either ordinary or nontoxic distilled water. The reactions to litmus paper of the solutions thus prepared were as follows: Sachs's, very slightly acid; Knop's, acid; Molisch's, slightly acid; Crone's, neutral. The salts used were, in every case, those of Merck.

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* These solutions contained salts in the following proportions:

The results obtained from the various cultures in these nutrient solutions are given in TABLE I, where each culture is denoted by a letter indicating the condition of the culture during the period of the experiment, the number of letters thus denoting, in each case, the number of times that the experiment in question was performed. The durations of the cultures are also given. The same notation is used in TABLES II, III, and V.

Using nontoxic distilled water, prepared in a manner to be described later, the best growth was shown with all the media in concentrations containing from 0.05 to 0.1 per cent of total salts. In these concentrations of the solutions of both Molisch and Crone, *Spirogyra* was kept growing vigorously, in apparently perfect condition, for a period of about two months, at the end of which time the experimentation was discontinued. Crone's solution seemed slightly more favorable to the organism than did that of Molisch; Sachs' solution produced a fair growth; while Knop's medium was distinctly unfavorable, probably because of the marked acidity, free acid being injurious to many algae (**10**). No doubt still other media might prove as favorable as those of Molisch and Crone.

When ordinary distilled water was used in preparing the solutions a good growth was never obtained, but the results in both Molisch's and Crone's solutions seemed slightly better in concentrations of from 0.5 to 1 per cent than in weaker solutions. This will be discussed later.

The addition of one per cent of agar to the nutrient solutions, the *Spirogyra* filaments being laid on the surface of the coagulated

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| SACHS | KNOP | MOLISCH | CRONE |
|---|---|---|---|
| 1 g. $KNO_3$, | 1 g. $Ca(NO_3)_2$, | 1 g. $(NH_4)_2HPO_4$, | 1 g. $KNO_3$, |
| 0.5 g. $NaCl$, | 0.25 g. $KNO_3$, | 0.5 g. $KH_2PO_4$, | 0.5 g. $MgSO_4$, |
| 0.5 g. $CaSO_4$, | 0.25 g. $MgSO_4$, | 0.5 g. $MgSO_4$, | 0.5 g. $CaSO_4$, |
| 0.5 g. $MgSO_4$, | 0.25 g. $KH_2PO_4$, | 0.5 g. $CaSO_4$, | 0.25 g. $Ca_3(PO_4)_2$, |
| 0.5 g. $CaHPO_4$, | 0.125 g. $KCL$, | Trace $FeSO_4$. | 0.25 g. $Fe_3(PO_4)_2$. |
| Trace $FeCl_2$. | Trace $FeCl_2$. | | |

medium in a covered Petri dish, produced slight improvement in all the 0.1 per cent solutions from ordinary distilled water. A good growth was obtained on similar agar plates prepared from 0.5 per cent solutions (in ordinary distilled water) according to the formula of Sachs, of Molisch, and of Crone.*

Since the solutions here employed were all prepared from water and salts, it will be expedient to present the following considerations under the two headings: (II) Water and (III) Salts.

## II. WATER

Many authors have found that tap water and distilled water are toxic to organisms, an excellent review of the literature of this subject being given by Livingston (12). Most workers have attributed the toxic effects of ordinary distilled water to small amounts of various metals—especially copper—taken by the water from the still and from the supply pipes. Lyon (19) and Bullot (6), however, found that water distilled in glass was markedly toxic, thus indicating that part of the poisonous action may be due to the presence of volatile substances. Lyon attributed this effect to small quantities of ammonia in the water. Similarly Livingston (12) showed that redistillation from glass to glass, while improving the quality of his ordinary distilled water, did not render it harmless. He obtained evidence indicating that part of the toxic bodies present in ordinary distilled water thus redistilled are volatile and reappear in the distillate, while part are nonvolatile and remain in the undistilled residue. The same writer prepared nontoxic water by shaking ordinary distilled water with highly absorbent solids, especially with purified lamp black, and then filtering out the solids. He concluded that the solids had absorbed, or at least removed from solution, both the volatile and the nonvolatile toxic substances.

In the present investigation both tap water and ordinary distilled water were found to be markedly toxic to *Spirogyra*, and were subjected to various treatments in an effort to obtain some evidence concerning the nature of the poisonous substances involved. The tap water was drawn from the faucet of the laboratory supply, directly into the culture dishes. The distilled water used was from the usual supply of the laboratory, obtained from a local druggist. It had been distilled in a copper still and kept in glass. All such water used in these experiments was from the same original supply and may thus be considered as uniform. The results obtained in the study of the physiological properties of various waters are given in TABLES II and III.

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* Knop's solution was so acid that 1 per cent agar would not harden in a 0.5 per cent concentration of the medium.

Tap water was usually fatal to the plant within a few days, although its toxicity varied slightly at different times (II, 1*). This water, when distilled with glass boiler and glass condenser, was as toxic as the untreated water (II, 2). The distillate was then sampled at different times during the run, by placing culture dishes at the outlet of the condenser and allowing them to receive the water directly; the alga was introduced into these water samples after they had cooled. Samples collected at the beginning and end of the run were as toxic as the untreated water (II, 3, 3*b*). About the middle of the run the distillate collected was found to be decidedly less injurious than the untreated tap water (II, 3*a*). Concentration of the tap water to one tenth its original volume by boiling (II, 4) and also by allowing evaporation to proceed at a temperature much below boiling, till only about a sixth of the original volume remained (II, 5, 6), produced marked improvement over the original untreated water, but concentration by evaporation to one third of the original volume did not improve its quality (II, 7). It will be noted, however, that the untreated water of this particular test was more toxic than in the two former experiments. In general, concentration seems to have improved the quality of the water, probably by driving off some of the volatile toxic substances, though it is of course not to be forgotten in this connection that the nonvolatile constituents were much more concentrated in the treated water than in the original.

The effect of high temperature without concentration was also tested. Heating tap water in the autoclave for 15 minutes, at a temperature of 144° C., destroyed all the toxic material, or else rendered it nontoxic; in water thus treated *Spirogyra* filaments remained in perfect condition for a month, while in untreated water drawn from the tap at the same time, nearly all the filaments succumbed within a few hours (II, 8). Heating tap water to a temperature of 100° C. in a steam sterilizer for 45 minutes produced no improvement; the filaments of *Spirogyra* died almost as quickly in this as in untreated water (II, 9).

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* Roman numerals followed by Arabic, in parentheses, refer to table and experiment, respectively.

Treatment of the tap water of the Heidelberg supply by the method of shaking with finely divided carbon, as employed with such marked effect by Livingston in the preparation of culture media from his ordinary distilled water, produced no improvement. In this treatment Merck's "animal charcoal" was employed as absorbing solid, without preliminary treatment of any sort. While it seems probable that there may be essential differences between the various forms of finely divided carbon now upon the market, as to their efficiency in the water treatments here considered, no comparisons have been made in this regard. The form here used was chosen merely because of its convenience.

Although, as has been pointed out, simple distillation of the Heidelberg tap water from glass into glass failed to correct the toxicity of the water, it was found that this same process of distillation rendered the water nontoxic to the *Spirogyra* here employed (II, 10) *when animal charcoal was present in the retort during the distillation.* In this treatment the boiler was a flask of Jena glass having a capacity of one liter; the condenser was of glass, provided with a bend to prevent the entrance of spray from the boiling liquid and thoroughly washed by use; and the receiver was a flask of Jena glass. No cork or rubber or material other than glass was used on the condenser. The usual charge was about 800 cc. of tap water with from five to ten grams of Merck's animal charcoal. Boiling was not allowed to become very violent and the process was stopped when the charge had been reduced to about one eighth of its original volume. In water thus prepared *Spirogyra* filaments remained in apparently healthy condition until the appearance of injury from lack of nutrient salts.

This method of distillation from animal charcoal showed some variation in the quality of the water produced (II, 10), but the differences exhibited were not as great as those obtained with different portions of water from the stock culture (III, 36). The water distilled from charcoal was frequently tested throughout the experiments,—by determining its effect, when used alone, upon *Spirogyra* filaments. Out of more than ten runs the distillate from but a single one was found to be decidedly toxic to the alga. The cause of this single failure was not investigated. The new method was uniformly employed for the preparation of nontoxic water throughout the course of these studies.

Several experiments were carried out to throw light upon the question of the manner of operation by which distillation from animal charcoal may produce such a marked effect upon the physiological properties of the Heidelberg water. Not only the distillate but also the undistilled and concentrated residue (after removal of the charcoal by filtration through paper) showed marked improvement as compared with the untreated tap water (II, 11, 12). Furthermore, a high degree of toxicity was produced in the practically nontoxic water, either of the filtered residue or of distillate, by the addition of a portion of the used charcoal that had been retained in the filter (II, 11*b*, 12*a*, 12*c*).

From the above observations it appears clear that the animal charcoal in the boiler of the still must have acted to remove toxic substances from the water, although, as has been stated, such action could not be detected at ordinary temperatures. It also appears that charcoal which has been the agent in this removal of injurious material becomes itself able to reproduce toxic properties in nontoxic water to which it may be added. It may be therefore considered as at least highly probable that the action of the finely divided carbon of these distillation experiments was that of an absorber rather than that of an oxidizing or other catalytic agent. Of course it is open to question, whether the toxicity reproduced in harmless water by used animal charcoal may be caused by the same substances as was the original toxicity of the tap water before treatment. The state of our knowledge of these matters precludes their further analysis at this time.

One experiment was made to show the relative resistance to the toxic influence in tap water of two portions of *Spirogyra* which were originally from the same source but which had been subjected for a time to different conditions. One sample was taken from the stock jar and another from a vigorous culture which was originally from the stock culture and then had grown for the last 68 days in 0.1 per cent Crone's solution. The two samples contained approximately equal numbers of filaments. They were well rinsed in nontoxic water and then placed together in untreated tap water in a covered glass dish. Within a few hours the filaments from the stock jar showed decided injury, and, at the end of six days, when the experiment was discontinued, about one third of them were dead (II, 13). The filaments transferred from Crone's solution showed no signs of injury during the period (II, 14). This experiment emphasizes the facts, well recognized, but not always considered by workers with cultures, that the internal conditions of organisms are fully as important in determining their behavior as are the conditions of the surroundings, and that the past history of organisms (the sort of surroundings under which they *have* lived) greatly influence their internal nature. Comparable results cannot be expected, in such work as this, unless the living material used has been kept under uniform conditions for a considerable time previous to experimentation.

Turning to the results obtained with ordinary distilled water (TABLE III), this was found to be extremely toxic, killing all the filaments of *Spirogyra*, except in a single instance,* in from one to nine days (III, 1). Redistilling this water in glass produced a decided improvement in some cases, but none in others (III, 2–6). Similarly, the samples of water obtained as above described, at the beginning, middle and end of the distillation run, varied greatly and inconsistently among themselves. The undistilled portion left in the boiler after redistillation had been stopped was always just as toxic as untreated water (III, 2*a*, 3*c*, 4*e*, 5*e*). Making from the distillate or from the undistilled residue (III, 4, 5) a 0.5 per cent concentration of Crone's solution produced no improvement, so that this addition of nutrient salts failed to counteract the toxicity of these two waters. The addition of animal charcoal to the boiler during redistillation in glass usually produced no improvement over the water redistilled without the solid (III, 7, 8). Furthermore, the undistilled residue from such redistillation (after removal of the carbon) showed no marked improvement. Boiling with or without carbon (III, 2–10) is thus seen to have produced no correction of the toxic conditions of ordinary distilled water. Heating in an autoclave for 15 minutes at 144° C. made ordinary distilled water decidedly less injurious, but did not render it entirely nontoxic (III, 11).

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* In this case an unusually small amount of distilled water was used, in proportion to the number of filaments in the culture.

Dilution with an equal volume of physiologically pure water (III, 12) or with water from a vigorous culture of *Nitella* (III, 13) did not make the ordinary distilled water less toxic, but dilution with an equal volume of a colloidal platinum solution (III, 14) rendered this water less injurious.*

The addition to ordinary distilled water, at the time of adding the alga, of filter paper (III, 15), cotton (III, 16), kaolin (III, 17), quartz sand (III, 18), or small amounts of chalk (III, 19, 20), produced no improvement; but the addition of abundant chalk (III, 21), lime (III, 25), broken agar sticks (III, 27), or dry sphagnum moss (III, 31) usually rendered the water nontoxic. *Spirogyra* filaments died within a few hours in ordinary distilled water to which a small amount of agar solution was added (III, 28), they lived nearly two months on the surface of a 1 per cent. solution of agar made with this same water (III, 29), but died within three days on the surface of a 2 per cent agar solution (III, 30). In ordinary distilled water shaken with chalk (III, 22), wood charcoal (III, 23), or garden soil (III, 24) and decanted after the subsidence of the solid, little or no improvement was observed at first, but decided improvement was shown after a few days. Addition to the water of sediment from a vigorous culture of *Nitella* (III, 32) produced better growth than that occurring in untreated water, while sediment from the stock culture of *Spirogyra* (III, 33) produced no improvement.

It was found that the alga filaments themselves may exert a profound influence upon the toxicity of the solution in which they are immersed. A transfer of filaments was made, in the usual manner, from the stock culture to a dish of ordinary distilled water (III, 34). These filaments were dead within eighteen hours. They were then removed from the culture and replaced by a new transfer of filaments from the stock culture. These also died within eighteen hours, and were replaced as before. The third transfer was killed in about two days, and the material which then replaced it lived fifteen days, but was in apparently poor condition at the end of that time and was replaced. The material of this fifth transfer preserved an apparently healthy condition for forty-four days, at which time the experiment was discontinued. The toxic water had been rendered nontoxic simply by treatment with alga filaments. A clearer example of the effects of plants in removing or counteracting the toxic substances of a solution would be difficult to imagine. Similar results were obtained by Dandeno (7) upon growing corn seedlings in toxic solutions of HCl and $H_2SO_4$.

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* This colloidal platinum solution was kindly prepared by Dr. W. Fraenkel in the laboratory of Prof. G. Bredig. It contained 0.0096 g. of platinum per 100 c.c.

Toxic water may be greatly improved for *Spirogyra* by previous treatment with another alga. A mass of *Ulothrix* (with a few *Spirogyra* filaments) was allowed to remain twenty-eight days in ordinary distilled water (III, 35), while a control of a similar dish with another sample of the same water, but without alga, stood beside the first. At the end of this period the *Ulothrix* was removed and *Spirogyra* from the stock culture was transferred to each of the two dishes. In the water which had been previously treated with *Ulothrix* the *Spirogyra* lived, albeit in rather poor condition, for twenty-two days, while in the untreated water of the control all filaments were killed within eighteen hours.

In connection with the above observations on the effectiveness of alga filaments in correcting the toxicity of ordinary distilled water, it should be noted that, in all these experiments, the amount of *Spirogyra* added, in proportion to the amount of medium employed, exerted a considerable influence upon the results. A large amount of the alga was able to live in a small amount of a decidedly toxic medium. In all such cases the filaments on the outside of the mass were killed while those within remained in good condition. Similar results have been obtained by Nägeli (20), Deherain and Demoussy (8), Bullot (6), Dandeno (7), and Bokorny (3). In the same connection, water from the stock culture of *Spirogyra*, tested by the method used in these experiments, allowed a good growth (III, 36), but seemed less favorable than the water from a vigorous culture of *Nitella* (III, 37).

Although some of the experiments which have been considered were not repeated as many times as might be desirable, the different experiments appear to corroborate each other, and seem to throw some light on the general nature of the toxic substances present in these waters. It will be noticed that the effect of distillation and of high temperature was often different in the case of tap water and in that of ordinary distilled water. Some of these differences are summarized in TABLE IV, where a plus sign indicates *improvement*, a minus sign indicates *no improvement*. When both signs occur, different tests were in disagreement.

The facts of TABLE IV indicate that the toxic materials present in the tap water and in the ordinary distilled water here studied were, in part at least, different substances; whatever may have been the nature of these substances those in the ordinary distilled water were mainly very resistant to heat.

As to the general characteristics of the toxic substances in the ordinary distilled water, there appears no reason for doubting that they were probably, for the most part, metallic in their nature, derived in some way from the distillation apparatus. This is the conclusion reached by many authors who have studied the toxicity of ordinary distilled waters since the time of Nägeli, and such supposition introduces no difficulty in the interpretation of the experiments that are reported here; it might be expected that metallic poisons, whether dissolved as salts or ions or existing merely in suspension, would not be very sensitive to correction by heat. The effect of redistillation might be to transfer a portion of the poisons from retort to receiver, thus leaving the distillate more or less toxic, and at the same time concentrating another portion (nonvolatile with steam and not sensitive to the temperatures employed) in the retort.

It has been shown by Livingston *et al.* (11), Livingston (12), Wheeler and Breazeale (37), Schreiner and Reed (27, 28, 29), Shorey (30, 31), and Lathrop (26), that certain organic substances present in various soil extracts are toxic to wheat seedlings. Since, as was pointed out by Livingston (12), tap water may be considered as a natural soil extract, the possibility is suggested that it may be toxic on account of poisons derived from the soil. At the same time, it is of course probable that the toxicity of the Heidelberg tap water here tested may have been due, to a greater or less extent, to substances having their origin in the supply pipes.

It may be supposed that somewhat complex poisons emanating from the soil would be largely volatile (either as such or as their decomposition products, *e. g.*, ammonia), and would thus be removed from the retort in the process of distillation. If such volatile material were still toxic and were retained in the receiver, the distillate would of course exhibit toxic properties. But the logical possibilities are here very numerous and a more detailed *a priori* consideration of this problem would be out of place without much more thorough experimentation than is now available.

The toxicity of the waters here dealt with was probably due, in every case, to more than one substance; different portions of tap water varied in their injurious effects, different portions of ordinary distilled water were differently affected by redistillation in glass, and other lines of evidence might be deduced from the present studies to indicate that both waters contained more than one kind of injurious material.

It appears highly probable that the beneficial effect on the ordinary distilled water of chalk, lime, agar, sphagnum moss, soil, and finely divided carbon, was due to their adsorptive action, a conclusion similar to that reached in the work of Nägeli (**20**), Breazeale (**5**), Livingston *et al.* (**11**), and Livingston (**12**). In some cases all the poisonous substances seem to have been removed from solution, for the treated water was entirely nontoxic. The nontoxic water obtained by the distillation of tap water from animal charcoal contained more dissolved electrolytic material than did the toxic water prepared by simple distillation, as was shown by conductivity measurements kindly made upon these waters by Dr. W. Fraenkel, but the total salt content of the charcoal-treated water was calculated to be less than 0.01 per cent, so that the removal of toxicity seems not to be related to an increase in salt content. Furthermore, similar decrease in the toxicity of the water was brought about by substances which were chemically very unlike the charcoal used in these distillations. The small amount of chalk that failed to produce improvement when added to ordinary distilled water was much in excess of the amount dissolved, so that the marked beneficial effect of a larger amount of chalk cannot be considered as due to the addition of calcium to the water. The improvement produced by colloidal platinum and by agar jelly appears also to have been due to adsorptive action, though a discussion of this matter will not be given place here. It is interesting to remark that addition of sand, cotton, and filter paper failed to produce any correction of the ordinary distilled water of these experiments, although these substances have been found by Nägeli (20), Dandeno (7), True and Oglevee (36) and Jensen (9), to be active in improving other toxic solutions. The difference thus noted may be of course due either to a difference in the toxic substances dealt with or to differences in the solids used. Breazeale (5) obtained similar negative results upon adding quartz flour, filter paper, paraffin, or sand to solutions of $H_2SO_4$ which were toxic to corn seedlings. He suggested that the failure of these substances to reduce the toxicity of the solutions was due to the high concentration of $H_2SO_4$ necessary to produce toxic action, and the consequent relatively slight adsorption of the acid by the solids. This explanation can not, however, apply to the results described above, since in these experiments the amounts of the toxic substances were extremely small.

In considering the behavior of *Spirogyra* in different nutrient media (see p. 335), it has been observed that the optimum concentration of the solution appeared to be a function of the kind of water used. With solutions that contained salts in the proportions adopted by Molisch and Crone and were prepared with non-toxic water, the optimum concentration for the growth of *Spirogrya* was found to be from 0.05 to 0.1 per cent, while similar solutions prepared with ordinary distilled water exhibited an optimum concentration ten times as great. It thus appears that the toxicity of the ordinary distilled water was more or less overcome by the larger amount of nutrient salts present and that, conversely, the retarding effect of relatively high salt concentration was counterbalanced by the presence of the toxic material of the water. The influence of dissolved salts in overcoming the toxicity of solutions has been noted by other authors,* but the whole question here raised is not at present in a state to warrant an attempt at detailed discussion. It is obviously related to the general problem of physiological antagonism. Some points on salt antagonism will be brought forward in the succeeding section.

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* Breazeale (4), Livingston *et al.* (11), Livingston (12), Sumner (35), Schreiner and Reed (28), and Schreiner and Skinner (32, 33).

The beneficial effect of the action of alga filaments, in rendering originally toxic water less toxic to other filaments of the same or another form (see p. 341–2), has been noted already. While the evidence at hand suggests the probability that this effect may be due to an absorptive (or adsorptive) action on the part of the filaments,—Nägeli (20), Deherain and Demoussy (8),—and that it may be therefore directly comparable with similar effects produced by other solids with very large surface exposure, it must be nevertheless remembered that such an effect may be also partially or wholly due to chemical alterations of the originally toxic substances, as if brought about by enzymatic or other material emanating from the plant. No evidence is available for a decision in this matter. The effects produced upon *Spirogyra* in toxic water, by sediment from the stock culture of this plant (III, 33) or from a *Nitella* culture (III, 32), together with the comparative growth of the alga in water from the two cultures just mentioned (III, 36, 37), may suggest a possible excretion from *Spirogyra* of material more harmful to itself than to *Nitella*. It appears that the question of toxic and antitoxic excretions may enter into problems of algal growth just as it has come to play so important a part in the physiology of the bacteria and fungi and, recently, in that of the higher plants.

## III. SALTS

It has been frequently shown for both animals and plants that the toxicity of many solutions of single salts may be counteracted by the presence of other salts in the same solution, without regard to the physiological effect of the latter salts when employed singly.

Loew (15, 16, 17, 18) would restrict this conception of the antagonistic action of two ions to the single case of calcium and magnesium. Benecke (1) showed that the toxicity of other salts than those of magnesium is counteracted by calcium, but ascribed the antagonistic action to calcium only. Osterhout (21, 22, 23, 24, 25) has, however, observed antagonism between fourteen different pairs of ions, including potassium, sodium, ammonium, calcium, magnesium, strontium, and barium. Loeb (13) reported that the toxic effect of a pure NaCl solution, on the eggs of *Fundulus*, was partly counteracted by each of a number of salts, including the bases: calcium, barium, magnesium, strontium, cobalt, aluminium, lead, zinc, and chromium.

The experimentation about to be reported, upon the relation to *Spirogyra* of the salt content of the medium, is merely qualitative, the aim being to gain some knowledge of a number of possible conditions in algal cultures rather than to investigate any of the chemical questions here encountered. No attention has been given to the chemical characteristics of the salts employed, the solutions having been prepared on the percentage basis, and the terminology of percentage will be retained in the discussions. The experiments are partly repetitions of those of Benecke (1). Aside from the effect of dissolved salts to counteract the toxicity of salt solutions, these experiments also include a number of tests bearing upon the influence of undissolved materials upon this same sort of toxicity.

Throughout these experiments no water was used which had not been shown to be nontoxic; it had all been distilled from animal charcoal in glass, condensed in glass, and collected in glass. Precautions were always taken to avoid possible contamination of the dishes used, especially in the case of the cultures without calcium. All salts used, except the $CaCO_3$, were those of Merck. The experiments are listed, with their results, in TABLE V.

For the study of the influence of calcium in the medium a calcium-free solution was prepared from equal portions by weight of $KNO_3$, $K_2HPO_4$, $KCl$, and $MgSO_4$. This solution was tested in two concentrations, 0.4 and 0.1 per cent (of total salt content by weight), and both proved highly injurious to the *Spirogyra* here employed (V, 1). The addition of calcium-free animal charcoal produced no improvement in either case (V, 2), but common wood charcoal, pulverized, which was shown by test to contain some calcium, improved the weaker solution (V, 3). In the weaker solution a small amount of $CaCl_2$ produced about as marked improvement as did wood charcoal (V, 4), a trace of $CaCO_3$ allowed still better growth (V, 5), while an abundance of the last-named, only slightly soluble salt kept the alga in excellent condition during the entire period of the experiment—35 days (V, 5). These same experiments, performed with solutions prepared from ordinary distilled water, gave the same results, except that the solutions thus made were all more toxic than those from nontoxic water.

A solution similar to the one without calcium, but with magnesium also absent, was prepared with equal parts by weight of $KNO_3$, $K_2HPO_4$, and KCl. This was more toxic than the calcium-free solution with magnesium present; both 0.4 and 0.1 per cent concentrations were fatal to the plant within two days (V, 6). Animal charcoal produced no improvement in the stronger solution (V, 7). The addition of calcium carbonate in considerable excess of its solubility enabled the plant to grow for several days (V, 8). A solution containing 0.1 per cent of the three potassium salts and 0.1 per cent of $CaCl_2$ was fatal within three days (V, 9), while the addition of 0.1 per cent of $MgSO_4$ to the 0.3 per cent solution of potassium salts prolonged the life of the organism for six days, but did not completely counteract the toxicity (V, 10). The last is a repetition of the first experiment of this section, where the organism lived 28 days (V, 1).

A simple solution of magnesium sulphate was fatal to the plant in concentrations ranging from 0.4 to 0.01 per cent (V, 11). Animal charcoal produced improvement only in the weakest of these concentrations (V, 12). Wood charcoal (with some calcium present) slightly improved both the 0.1 and the 0.4 per cent solutions (V, 13). While the simple solution of $MgSO_4$ killed the organism in two or three days, a 0.4 or 0.1 per cent solution of $MgSO_4$ plus 0.1 per cent of $CaCl_2$ (V, 14), as well as 0.1 per cent solution of $MgSO_4$ plus 0.1 per cent of KCl (V, 15), allowed the alga to live fourteen days. In these experiments the addition of potassium was just as efficient in counteracting the toxicity of magnesium as was the addition of calcium.

To determine whether the effect of $CaCO_3$ in counteracting the toxicity of $MgSO_4$ is to be considered as due solely to the addition of calcium to the solution or partially to other causes, the following experiments were carried out. A saturated solution of $CaCO_3$ was prepared and to this was added 0.4 per cent of $MgSO_4$. At the same time, to a 0.4 per cent solution of $MgSO_4$ was added an excess of finely divided $CaCO_3$. In the former solution, the alga was markedly injured within nineteen hours, and was totally dead at the end of fourteen days (V, 16). In the solution containing solid $CaCO_3$, however, the alga remained in good condition for seven days and had many filaments apparently uninjured at the end of fifteen days, when the experiment was discontinued (V, 17). These and other lines of evidence obtained during the progress of this work seem to indicate that the beneficial effect of the powdered $CaCO_3$ was due in part to other causes than the mere addition of calcium to the solution, probably to its adsorptive action. This suggestion is further supported by the results of Livingston *et al.* (11) Snyder and Cook (34), Breazeale (5), and Schreiner and Reed (28).

The colloidal solution of platinum already referred to rendered a 0.01 per cent solution of $MgSO_4$ decidedly less toxic (V, 18), but the addition of 0.008 per cent $PtCl_4$ to a 0.01 per cent $MgSO_4$ solution produced no observable improvement.

Although, as was noted above, the addition of 0.1 per cent of KCl rendered a 0.1 per cent solution of $MgSO_4$ less toxic, a 0.1 per cent solution of KCl alone was decidedly more toxic than this mixture (V, 20), and a 0.4 per cent solution of KCl was fatal within two days (V, 19). This result is in agreement with the work of Osterhout (22). The colloidal solution of platinum produced improvement in a 0.1 per cent solution of KCl (V, 21). Addition of 0.1 per cent of $CaCl_2$ to a 0.1 per cent solution of KCl rendered the latter slightly less toxic (V, 22), although a 0.2 per cent solution of $CaCl_2$ was fatal within a few hours or days (V, 23).

Two experiments were made bearing upon the relative endurance, in solutions of the above salts, of samples of *Spirogyra* originally from the same source, but subjected for a time to different surroundings. One portion of an alga culture which had been growing vigorously for sixty-six days in 0.1 per cent Crone's solution was rinsed in nontoxic water and placed in a solution containing 0.1 per cent of KCl and 0.1 per cent of $CaCl_2$ (V, 24) and another portion of the same culture was likewise rinsed and placed in a 0.2 per cent solution of $CaCl_2$ (V, 25). On the following day were added to both of these cultures samples of *Spirogyra* from the stock jar. In both cases the solution was injurious to both samples of the alga, but decidedly less so to the filaments from Crone's solution than to those from the stock culture (V, 22, 23). This result again emphasizes the well-known fact, already mentioned, that the internal conditions or the physiological state of organisms used in experimentation of this sort must always be taken into account.

In this portion of the work, no evidence was obtained for a specific action of the calcium salts in antagonizing other salts. The action of $CaCl_2$ in antagonizing $MgSO_4$ and KCl was no more pronounced than was that of KCl in counteracting the toxicity of $MgSO_4$ and of $CaCl_2$, and that of $MgSO_4$ in counteracting the toxicity of KCl and of $CaCl_2$. These results seem strongly to support the conception of Loeb (14) and Osterhout (22, 25) of the importance of physiologically balanced solutions. $MgSO_4$, $CaCl_2$, and KCl, when used separately, and a mixture of three potassium salts were all extremely toxic in the present investigation, but when the salts of any two of these bases were brought together (as in combinations of $MgSO_4$ and $CaCl_2$, $MgSO_4$ and KCL, KCl and $CaCl_2$) the resulting solution was much less toxic. A mixture of the three potassium salts here employed was as toxic as KCl alone and only when salts of all three metals were present in favorable proportions was good growth obtained. Results similar to these were reported by Bokorny (2). As it has been shown by several workers that *Spirogyra* requires calcium, these facts cannot be interpreted by supposing that this alga differs from higher plants in being able to survive in the absence of this element.

The improvement produced in the weaker toxic salt solutions by animal charcoal may be considered as probably due to the adsorptive action of the finely divided carbon. The beneficial effect of the addition of colloidal platinum to toxic solutions may have been likewise due to its adsorption of the toxic salts.

The author is gratefully indebted to Prof. B. E. Livingston for helpful suggestions in the preparation of this paper.

## SUMMARY

1. Cultures of *Spirogyra longata* (Vauch.) Kg. were kept under laboratory conditions in a vigorous state during the entire period of the experiments,—more than 60 days. Crone's solution was the best of the nutrient solutions tested, and that of Molisch was almost as satisfactory, but the solution of Sachs seemed slightly

less favorable, while that of Knop was distinctly unfavorable, probably because of its marked acidity.

2. Both the tap water and the ordinary distilled water of the Heidelberg Institut were markedly toxic to this *Spirogyra*, even after distillation (or redistillation) from glass to glass.

3. The toxicity of the tap water was partially removed by concentrating the water to a fraction of its original volume, and was entirely removed by heating to 144° C. or by distillation in glass from animal charcoal; but the water was not greatly improved by simple distillation or by heating to 100° C. The toxicity of ordinary distilled water was partly or wholly corrected by the presence in the culture of chalk, lime, solid agar, dry sphagnum moss, colloidal platinum, or of other adsorbents; it was partially corrected by redistillation or by heating to 144° C. The presence in the cultures of filter paper, cotton, sand, kaolin, or $CaCl_2$ was without effect, and redistillation in glass from animal charcoal did not produce nontoxic water.

4. The results obtained seem to indicate that the toxic materials present in the tap water were mostly volatile (perhaps organic substances derived from the soil), with a relatively small amount of nonvolatile material probably emanating from the supply pipes. The toxic substances in the ordinary distilled water were mostly nonvolatile (probably derived from the supply pipes and from the still), with a small amount of volatile matter carried from retort to receiver with the distillate.

5. When nontoxic water was used, the best growth was obtained in nutrient solutions containing from 0.05 to 0.1 per cent of total salts; but when ordinary distilled water was used, the optimum concentration of similar nutrient solutions was ten times as great.

6. With $KNO_3$, $K_2HPO_4$, KCl, $MgSO_4$, and $CaCl_2$ (singly or in various combinations), a mixture of the three potassium salts was as toxic as KCl alone; KCl, $MgSO_4$, and $CaCl_2$ were all extremely toxic when used singly; but mixtures of any two of these three were less toxic than the solution of a single one. Good growth was obtained only when salts of all three metals were present in favorable proportions. The toxicity of $MgSO_4$ appeared to be as completely counteracted by KCl as by $CaCl_2$.

7. Weak solutions of certain toxic salts were improved by the addition of calcium-free animal charcoal or colloidal platinum. It seems indicated that the effect of powdered $CaCO_3$, in counteracting toxicity due to nutrient salts, was due in part to the adsorptive action of the solid.

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## TABLE I

CULTURES OF SPIROGYRA IN NUTRIENT MEDIA PREPARED WITH DISTILLED WATER

| No. of culture | Nature of culture medium | | | Duration of cultures, *days* | Condition of plants * |
|---|---|---|---|---|---|
| | Author and concentration, *per cent of total salts in medium* | Kind of distilled water used in medium | Agar added to medium, *per cent by weight* | | |
| 1 | Sachs, 0.05 | ordinary | — | 3 | *D* |
| 2 | 0.05 | nontoxic | — | 48 | *E* |
| 3 | 0.10 | ordinary | — | 10, 20 | *Pd, Pd* |
| 4 | 0.10 | do. | 1.0 | 34 | *P* |
| 5 | 0.10 | nontoxic | — | 40, 18, 44 | *E, Eg, Gp* |
| 6 | 0.50 | ordinary | — | 3,14 | *D, D* |
| 7 | 0.50 | do. | 1.0 | 60 | *E* |
| 8 | 0.50 | nontoxic | — | 50 | *G* |
| 9 | 1.00 | ordinary | — | 2 | *D* |
| 10 | 1.00 | nontoxic | — | 44 | *P* |
| 11 | Knop, 0.05 | ordinary | — | 3, 3 | *D, D* |
| 12 | 0.05 | nontoxic | — | 48 | *Gp* |
| 13 | 0.10 | ordinary | — | 3 | *D* |
| 14 | 0.10 | do. | 1.0 | 49 | *Pd* |
| 15 | 0.10 | nontoxic | — | 18, 31, 44, 11 | *G*,† *Pd, Pd, Pd* |
| 16 | 0.50 | ordinary | — | 18, 49, 1 | *G*,† *P, D* |
| 17 | 0.50 | nontoxic | — | 23, 2 | *Pd, D* |
| 18 | 1.00 | ordinary | — | 9 | *Pd* |
| 19 | 1.00 | nontoxic | — | 1 | *D* |
| 20 | Molisch, 0.05 | ordinary | — | 3 | *D* |
| 21 | 0.05 | nontoxic | — | 48 | *E* |
| 22 | 0.10 | ordinary | — | 7 | *Pd* |
| 23 | 0.10 | do. | 1.0 | 49 | *P* |
| 24 | 0.10 | nontoxic | — | 62, 62, 40, 40 | *E, G, G, G* |
| 25 | 0.50 | ordinary | — | 55, 48, 22, 14 | *G, P, P, Pd* |
| 26 | 0.50 | do. | 1.0 | 66 | *E* |
| 27 | 0.50 | nontoxic | — | 50 | *Eg* |
| 28 | 1.00 | ordinary | — | 46 | *P* |
| 29 | 1.00 | nontoxic | — | 51 | *P* |
| 30 | Crone, 0.05 | ordinary | — | 3 | *D* |
| 31 | 0.05 | nontoxic | — | 48 | *E* |
| 32 | 0.10 | ordinary | — | 3 | *D* |
| 33 | 0.10 | do. | 1.0 | 18 | *Pd* |
| 34 | 0.10 | nontoxic | — | 23, 40, 40, 51 | *E, E, E, G* |
| 35 | 0.50 | ordinary | — | 48, 13, 14 | *Eg*,† *Pd, Pd* |
| 36 | 0.50 | do. | 1.0 | 60 | *E* |
| 37 | 0.50 | nontoxic | — | 50 | *P* |
| 38 | 1.00 | ordinary | — | 82 | *G* |
| 39 | 1.00 | nontoxic | — | 51 | *Gp* |

* The condition of the plants is indicated as follows: *E*, excellent, *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters—as, *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters; thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† The amount of medium used was unusually small, in comparison with the number of filaments in the culture.

## TABLE II

CULTURES OF SPIROGYRA IN TAP WATER, WITH TREATMENTS AND ADDITIONS

| No. of culture | Treatments of, and additions to, tap water | Duration of cultures, *days* | Condition of plants* |
|---|---|---|---|
| 1 | Untreated | 54, 4, 1 | *P, D, D* |
| 2 | Distillate from glass to glass | 12, 24, 2 | *P, Pd, D* |
| 3 | As 2, first portion | 3, 2 | *P, D* |
| 3*a* | As 2, middle portion | 3, 12 | *Gp, Gp* |
| 3*b* | As 2, final portion | 12, 3 | *P, D* |
| 4 | Boiled to 1/10 orig. vol. | 15, 12 | *Eg, P* |
| 5 | Evaporated to 1/7 orig. vol. | 1 | *G* |
| 5*a* | Untreated (control to 5) | 1 | *P* |
| 6 | Evaporated to 1/6 orig. vol. | 1 | *Gp* |
| 6*a* | Untreated (control to 6) | 1 | *Pd* |
| 7 | Evaporated to 1/3 orig. vol. | 1 | *D* |
| 7*a* | Untreated (control to 7) | 1 | *D* |
| 8 | 15 mins. at 144° C. | 37 | *E* |
| 8*a* | Untreated (control to 8) | 37 | *Pd* |
| 9 | 45 mins. at 100° C. | 46 | *Pd* |
| 10 | Distillate from animal charcoal, glass to glass | 1 to 44 | *E, E, E, E, Eg, Eg, Eg, Eg, G, P* |
| 11 | Boiled with animal charcoal to 1/8 orig. vol., filtered | 26 | *Eg* |
| 11*a* | Distillate from 11 | 26 | *E* |
| 11*b* | Do., charcoal from 11 added to culture. | 26 | *Gp* |
| 12 | As 11, filtered | 16 | *G* |
| 12*a* | As 11, not filtered | 16 | *Gp* |
| 12*b* | As 11*a* | 16 | *G* |
| 12*c* | As 11*b* | 16 | *Gp* |
| 13 | Untreated plants from stock jar† | 6 | *G* |
| 14 | As 13, but plants from Crone's sol.† | 6 | *E* |

* The condition of the plants is indicated as follows: *E*, excellent; *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters, as: *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters; thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† Nos. 13 and 14 were simultaneous, in the same dish.

## TABLE III

CULTURES OF SPIROGYRA IN ORDINARY DISTILLED WATER, WITH TREATMENTS AND ADDITIONS

| No. of culture | Treatments | Additions | Duration of cultures, *days* | Condition of plants* |
|---|---|---|---|---|
| 1 | Untreated | — | 54, 9, 6, 1 | *Gp, D, D, D* |
| 2 | Distillate, glass to glass | — | 8 | *G* |
| 2*a* | Boiled, concentrated, residue from 2 | — | 1 | *D* |
| 3 | Distillate, glass to glass, to 1/8 orig. vol., first portion | — | 1 | *D* |
| 3*a* | As 3, middle portion | — | 4 | *Eg* |
| 3*b* | As 3, final portion | — | 4 | *G* |
| 3*c* | Boiled, contentrated to 1/8 orig. vol., residue from 3 | — | 1 | *D* |
| 4 | Distillate, glass to glass, to 1/9 orig. vol., first portion | — | 9 | *G* |
| 4*a* | As 4, middle portion | — | 9 | *P* |
| 4*b* | As 4, final portion | — | 9 | *P* |
| 4*c* | As 4, all portions mixed | — | 9 | *P* |
| 4*d* | Distillate, glass to glass | Salts to form 0.5 *p.c.* Crone's sol. | 9 | *D* |
| 4*e* | Boiled, concentrated, residue from 4*b* | — | 9 | *D* |
| 4*f* | As 4*e* | Salts to form 0.5 *p.c.* Crone's sol. | 1 | *D* |
| 5 | Distillate, glass to glass, to 1/16 orig. vol., 1st portion | — | 12 | *Eg* |
| 5*a* | As 5, middle portion | — | 12 | *Pd* |
| 5*b* | As 5, final portion | — | 12 | *D* |
| 5*c* | As 5, all portions mixed | — | 12 | *P* |
| 5*d* | Distillate, glass to glass | Salts to form 0.5 *p.c.* Crone's sol. | 12 | *Gp* |
| 5*e* | Boiled, concentrated, residue from 5*b* | — | 12 | *P* |
| 5*f* | As 5*e* | Salts to form 0.5 *p.c.* Crone's sol. | 1 | *D* |
| 6 | Distillate, glass to glass, first portion | — | 4 | *Pd* |
| 7 | Distillate from animal charcoal, glass to glass | — | 46 | *E* |
| 8 | As 7, to 1/8 orig. vol., first portion | — | 2, 12 | *D, D* |
| 8*a* | As 8, middle portion | — | 3, 12 | *G, Pd* |
| 8*b* | As 8, final portion | — | 12, 1 | *P, D* |
| 8*c* | Boiled with animal charcoal, filtered, concentrated residue from 8*b* | — | 3, 12 | *P, P* |
| 9 | Boiled 3 mins. | — | 1 | *D* |
| 10 | Boiled with animal charcoal, not filtered | — | 1 | *D* |
| 11 | 15 mins. at 144° C. | — | 39 | *G* |
| 12 | Untreated | Nontoxic water to double vol. | 2, 1, 1 | *D, D, D* |
| 13 | *do.* | *Nitella* water to double vol. | 1 | *D* |

TABLE III, *continued.*

| No. of culture | Treatments | Additions | Duration of cultures, *days* | Condition of plants* |
|---|---|---|---|---|
| 14 | *do.* | Colloidal Pt sol. to double vol. | 26 | *Gp* |
| 15 | *do.* | Filter paper | 10, 1 | *Pd, D* |
| 16 | *do.* | Cotton | 1, 2 | *D, D* |
| 17 | *do.* | Kaolin | 1, 1 | *D, D* |
| 18 | *do.* | Quartz sand | 1, 1 | *D, D* |
| 19 | *do.* | Chalk, trace | 2 | *D* |
| 20 | *do.* | *do.*, excess | 5 | *D* |
| 21 | *do.* | *do.*, abundance | 23, 56 | *E, Eg* |
| 22 | Shaken with chalk, decanted after 3 hours | — | 4 | *D* |
| 22*a* | As 22, decanted after 10 days | — | 9, 10 | *G, Pd* |
| 23 | Shaken with wood charcoal, decanted | — | 53 | *P* |
| 23*a* | As 23, decanted after 4 days | — | 39 | *G* |
| 24 | Shaken with soil, decanted after several days | — | 44 | *Gp* |
| 25 | Untreated | Lime, abundance | 2 | *E* |
| 26 | *do.* | $CaCl_2$, O 0.4 *p.c.* | 3 | *D* |
| 27 | *do.* | Agar sticks | 20 | *E* |
| 28 | *do.* | Agar, not hardening | 1 | *D* |
| 29 | *do.* | Agar, 1 *p.c.*, alga on surf. | 54 | *G* |
| 30 | *do.* | Agar, 2 *p.c.*, as 29 | 3 | *D* |
| 31 | *do.* | Sphagnum moss | 23, 23, 9 | *E, E, Pd* |
| 32 | *do.* | *Nitella* sediment | 48 | *Gp* |
| 33 | *do.* | *Spirogyra* sediment | 5 | *D* |
| 34 | *do.* |  | 1 | *D* |
| 34*a* | Water from 34, alga removed | Alga replaced by fresh material from stock jar | 1 | *D* |
| 34*b* | Water from 34*a*, alga removed | *do.* | 2 | *D* |
| 34*c* | Water from 34*b*, alga removed | *do.* | 15 | *P* |
| 34*d* | Water from 34*c*, alga removed | *do.* | 44 | *E* |
| 35 | Treated with *Ulothrix*,† removed after 28 days | *do.* | 22 | *P* |
| 35*a* | Untreated (control to 35) | — | 1 | *D* |
| 36 | Water from stock *Spirogyra* culture | — | 52, 47, 9, 4 | *E, Eg, G, Gp* |
| 37 | Water from *Nitella* culture | — | 52, 9 | *E, E* |

* The condition of the plants is indicated as follows: *E*, excellent; *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters—as, *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters; thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† *Ulothrix* killed except in center of mass.

## TABLE IV

EFFECT OF VARIOUS TREATMENTS OF TAP AND DISTILLED WATER UPON THEIR TOXICITY

| Treatment | Tap Water | Ordinary Distilled Water |
|---|---|---|
| Distilled in glass (distillate) | − | −+ |
| Distilled in glass (undistilled residue) | + | − |
| Distilled with carbon (distillate) | + | −+ |
| Distilled with carbon (undistilled residue) | + | − |
| Heated to 144° C. | + | +* |

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* Still toxic, but much improved.

## TABLE V

CULTURES OF SPIROGYRA IN SALT SOLUTIONS PREPARED FROM NONTOXIC WATER, WITH AND WITHOUT ADDITIONS

| No. of culture | Salts in solution, per cent | | | | Additions | Duration of culture, days | Condition of plants* |
|---|---|---|---|---|---|---|---|
| | $KNO_3$ | $K_2HPO_4$ | $MgSO_4$ | KCl | | | |
| 1† | 0.1 | 0.1 | 0.1 | 0.1 | — | 13, 28, 8 | *Gp, Gp, Pd* |
| 1*a* | 0.025 | 0.025 | 0.025 | 0.025 | — | 26, 29 | *Gp, P* |
| 2 | 0.1 | 0.1 | 0.1 | 0.1 | Animal charcoal‡ | 11 | *Pd* |
| 2*a* | 0.025 | 0.025 | 0.025 | 0.025 | *do.* | 26 | *Gp* |
| 3 | 0.1 | 0.1 | 0.1 | 0.1 | Wood charcoal¶ | 15 | *Pd* |
| 3*a* | 0.025 | 0.025 | 0.025 | 0.025 | *do.* | 35 | *G* |
| 4 | *do.* | *do.* | *do.* | *do.* | $CaCl_2$ | 28 | *G* |
| 5 | *do.* | *do.* | *do.* | *do.* | $CaCO_3$, trace | 35 | *Eg* |
| 5*a* | *do.* | *do.* | *do.* | *do.* | $CaCO_3$ abundance | 35 | *E* |
| 6 | 0.133 | 0.133 | — | 0.133 | — | 2 | *D* |
| 6*a* | 0.033 | 0.033 | — | 0.033 | — | 2 | *D* |
| 7 | 0.133 | 0.133 | — | 0.133 | Animal charcoal‡ | 3 | *D* |
| 8 | *do.* | *do.* | — | *do.* | $CaCO_3$ | 6 | *P* |
| 9 | 0.1 | 0.1 | — | 0.1 | $CaCl_2$ 0.1 *p.c.* | 3 | *D* |
| 10 | *do.* | *do.* | 0.1 | *do.* | — | 6 | *Pd* |
| 11 | — | — | 0.4 | — | — | 3, 2 | *D, D* |
| 11*a* | — | — | 0.1 | — | — | 3, 2 | *D, D* |
| 11*b* | — | — | 0.05 | — | — | 2 | *D* |
| 11*c* | — | — | 0.01 | — | — | 6, 2 | *Pd, D* |
| 12 | — | — | 0.4 | — | Animal charcoal‡ | 2 | *D* |
| 12*a* | — | — | 0.1 | — | *do.* | 2 | *D* |
| 12*b* | — | — | 0.05 | — | *do.* | 2 | *D* |
| 12*c* | — | — | 0.01 | — | *do.* | 9, 5 | *P, P* |
| 13 | — | — | 0.4 | — | Wood charcoal¶ | 15 | *P* |
| 13*a* | — | — | 0.1 | — | *do.* | 6 | *Pd* |
| 14 | — | — | 0.4 | — | $CaCl_2$ 0.1 *p.c.* | 15 | *P* |
| 14*a* | — | — | 0.1 | — | *do.* | 14 | *Pd* |
| 15 | — | — | *do.* | 0.1‖ | — | 14 | *P* |
| 16 | — | — | 0.4 | — | $CaCO_3$ to saturation | 14 | *Pd* |
| 17 | — | — | *do.* | — | $CaCO_3$, in excess | 15 | *Gp* |
| 18 | — | — | 0.01 | — | Colloid. sol. Pt 90 *p.c.* | 19 | *Gp* |
| 18*a* | — | — | *do.* | — | $Pt\ Cl_4$ 0.008 *p.c.* | 2 | *D* |
| 19 | — | — | — | 0.4 | — | 2 | *D* |
| 20 | — | — | — | 0.1 | — | 6 | *Pd* |
| 21 | — | — | — | *do.* | Colloid. sol. Pt | 12 | *P* |
| 22 | — | — | — | *do.* | $CaCl_2$ 0.1 *p.c.* | 7, 6 | *Gp,*° *Pd* |
| 23 | — | — | — | — | $CaCl_2$ 0.2 *p.c.* | 7, 1 | *D,*Δ *D* |
| 24 | — | — | — | 0.1 | $CaCl_2$ 0.1 *p.c.* | 7 | *G*° |
| 25 | — | — | — | — | $CaCl_2$ 0.2 *p.c.* | 7 | *G*Δ |

* The condition of the plants is indicated as follows: *E*, excellent; *G*, good; *P*, poor; *D*, dead. A combination of letters denotes a condition between that denoted by each of these letters—as, *Eg* indicates a condition between excellent and good, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the preceding column (duration in days) refer to the experiments in the same order as do the letters, thus, the first letter denoting *condition* and the first number denoting *duration* both refer to the same experiment, etc.

† Culture 23 days old showed no calcium with acetic acid and ammonium oxalate.

‡ A culture 14 days old showed no calcium.

¶ Containing some calcium.

‖ Showed no calcium.

° *Spirogyra* from culture in Crone's solution, 66 days old, was used in 24 and 25. The first test of the stock material (22) and of material from Crone's solution (24) were simultaneously carried out in the same dish.

Δ The second test of the stock *Spirogyra* (23) and of material from Crone's solution (25) were simultaneously carried out in the same dish.

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